

EPA/OPP MICROBIOLOGY LABORATORY  
ESC, Ft. Meade, MD

Standard Operating Procedure  
for  
Calibration of Eppendorf Pipettes Using the PCS 2 Pipette Calibration System

SOP Number: QC-19-02

Date Revised: 10-15-02

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Controlled Copy No.: \_\_\_\_\_

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### 1.0 SCOPE AND APPLICATION:

1.1 This SOP describes the procedure for calibrating pipettes using the PCS 2 Pipette Calibration System. Results are traceable to NIST.

2.0 DEFINITIONS:

2.1 NIST = National Institute of Standards and Technology

3.0 HEALTH AND SAFETY:

3.1 All kit reagents are non-hazardous aqueous based and may be disposed of by flushing down a drain.

4.0 CAUTIONS:

4.1 Make sure the PCS Instrument is kept out of the direct sunlight and away from anything hot or cold. The pipettes, the instrument, and the reagent kit should be stored at room temperature.

4.2 To ensure proper sample mixing, place the PCS Instrument on a sturdy table.

4.3 Do not open the PCS Vial Holder Cover until you are prompted with "Pipette Sample #" or "Remove Cap and Insert Blank."

5.0 INTERFERENCES: Issues discussed in 5.1 through 5.8 must be recognized to avoid interference during the calibration process. Interference could lead to erroneous readings.

5.1 Ensure that the reagent kit is at the same ambient temperature as the instrument by storing them in close proximity to each other (in the same laboratory at room temperature).

5.2 Once the blank reagent vial has been inserted into the instrument it must not be turned, moved, or removed until the entire pipette calibration procedure has been completed, or the vial has been consumed. If the vial is removed and sample solution has been pipetted into the vial, this vial is no longer usable.

5.3 Blank reagent vials should be moved immediately from the vial block into

the vial holder, giving them no chance to warm or cool along the way.

- 5.4 Always take a fresh aliquot of sample solution if the solution has been in the sample aliquot container for over one-half hour.
- 5.5 Always place the cap (lightly) on the aliquot containers between pipette sampling to minimize reagent evaporation.
- 5.6 The PCS reagent kit should be stored at room temperature. Avoid storage temperatures above 32°C (90°F). Kit components should be kept in the dark to the extent practical.
- 5.7 To ensure that accurate and precise results are obtained during pipette calibrations and instrument calibration checks, all vials should be free of fingerprints or smudges prior to placement into the vial block. **THUS, AVOID HANDLING THE LOWER PORTION OF THE VIALS.** Fingerprints or smudges on the vials that fall into the optical path will introduce errors that cause inaccurate readings. In the case of instrument calibration checks, false failures may occur. All vials are cleaned and inspected prior to shipping. If the lower portion of the vial is inadvertently touched, it should be properly cleaned prior to use. The following procedure is recommended for cleaning all PCS vials:
  - 5.7.1 Obtain rubbing alcohol (isopropyl) and pour into a small container. Pour enough rubbing alcohol into the container so as to cover approximately 1 inch of the vial when immersed in the alcohol.
  - 5.7.2 Holding the vial by the cap, immerse (approximately 1 inch) in the alcohol.
  - 5.7.3 Remove the vial from the alcohol and, using a lint free wipe (e.g., Kimwipe), wipe the vial dry by rotating the vial in the lint free wipe several times.
- 5.8 The power line to which the instrument is connected should be free of voltage variations caused by other equipment such as water baths or refrigerators.

## 6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

## 7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 ARTEL PCS 2 Pipette Calibration System Instrument, Serial No. 6250.
- 7.2 Eppendorf Series 2000 Reference Adjustable-Volume Pipettes (two of each model)
  - 7.2.1 Model 22 47 030-2, 100-1000  $\mu$ L Serial no. 296002 and 303326
  - 7.2.2 Model 22 47 025-6, 50-200  $\mu$ L Serial no. 106530 and 106535
  - 7.2.3 Model 22 47 005-1, 0.5-10  $\mu$ L Serial no. 157670 and 152348

## 8.0 INSTRUMENT OR METHOD CALIBRATION:

- 8.1 The PCS Instrument calibration should be checked monthly. To check the instrument calibration, an instrument calibrator kit is used. It contains four vials of different concentrations of mixed sample and blank reagents and an insert with calibrator values, lot code, expiration date and instructions. The calibrator values contain information about these concentrations derived from a reference instrument at the factory which itself has been calibrated using standards traceable to NIST. The PCS instrument calibration check printout will be copied (it is printed on thermal paper which fades over time) and stored in the Pipette Calibration Log Book.
- 8.2 Between uses, the calibration kit should be stored in darkness at room temperature (18-28°C) to allow for a six month shelf life.
- 8.3 PCS calibrator kit certificates of calibration will be stored in the Pipette Calibration Log Book.
- 8.4 PCS reagent kit certificates of calibration will be stored in the Pipette Calibration Log Book.
- 8.5 Refer to Chapter 5 of the PCS Procedure Guide for detailed instructions

and pass/fail specifications.

9.0 SAMPLE HANDLING AND STORAGE: Not applicable

10.0 PROCEDURE AND ANALYSIS:

10.1 Pipette calibration will be performed monthly when in use on the 50-200  $\mu\text{L}$  pipettes (model 22 47 025-6; serial no. 106530 and 106535) and the 100-1000  $\mu\text{L}$  pipettes (model 22 47 030-2; serial no. 296002 and 303326) and will be good for one month from the date of calibration. Calibration will be performed on a weekly basis for 0.5-10  $\mu\text{L}$  pipettes (model 22 47 005-1, serial no. 157670 and 152348) when they are in use and will be good for one week from the date of calibration (See Table 1). Pipettes will be calibrated using the PCS 2 Pipette Calibration System according to the following instructions:

Table 1

Pipette Calibration Summary					
Model	Serial No.	Volume Range	Cal. Volume(s)	Replicates	Cal. Interval
22 47 030-2	296002	100-1000 $\mu\text{L}$	100, 500 and 1000 $\mu\text{L}$	2	Monthly, when in use
22 47 030-2	303326	100-1000 $\mu\text{L}$	100, 500, and 1000 $\mu\text{L}$	2	
22 47 025-6	106530	50-200 $\mu\text{L}$	50, 100 and 200 $\mu\text{L}$	2	Monthly, when in use
22 47 025-6	106535	50-200 $\mu\text{L}$	50, 100 and 200 $\mu\text{L}$	2	
22 47 005-1	157670	0.5-10 $\mu\text{L}$	10 $\mu\text{L}$	5	Weekly when in use
22 47 005-1	152348	0.5-10 $\mu\text{L}$	10 $\mu\text{L}$	5	

10.2 Turn on the PCS Instrument and the printer.

10.3 Blank vials should be loaded into the vial block at least 15 minutes prior to their use. Four can be loaded into the vial block at this time. Inspect for

smudges and clean them if necessary (see sect. 5.7).

- 10.4 Allow the instrument to self calibrate and follow the on-screen instructions. Enter a pre-assigned Operator ID number. Due to differences in operator technique, the analyst planning to use the pipette should also perform the calibration. If no significant difference in pipette volumes between analysts is detected over time, it will not be necessary for the analyst calibrating the pipette to be the only one who uses it during the calibration interval (see Table 1 for calibration intervals).
- 10.5 When prompted, enter the four or five digit lot code shown on the reagent kit insert and on the exterior label affixed to the top of the reagent kit. Enter the lot information if this is a new lot of reagents.
- 10.6 After self calibration, the instrument will prompt the operator to insert the CAL A Vial.

IMPORTANT: The CAL A calibration standard should always be inserted into the vial holder with the CAL A label facing toward the front of the PCS. Be careful not to smudge or get fingerprints on the lower portion of the vial as this is a part of the optical path and any smudges will decrease the accuracy of the pipette calibration results (see sect. 5.7). Never remove the cap of a calibration standard.

- 10.7 After closing the cover on the vial holder, the PCS will perform a zero check. Once the zero check is complete, remove the CAL A vial and replace it in the Reagent Kit.
- 10.8 Remove a blank vial from the block. Invert it several times to mix the contents thoroughly, remove the cap and set it aside.

IMPORTANT: Each blank reagent vial must be handled with care. The vial contains a predetermined amount of liquid and any spillage will lead to inaccurate results. The lower portion of the vial is a part of the optical path and smudges will decrease the accuracy of the calibration results. Touching it should be avoided (see sect. 5.7).

- 10.9 Open the vial holder cover and insert the blank reagent vial into the vial holder located under the vial holder cover. Close the cover. The Instrument will take a reading of the blank.

**IMPORTANT:** Once the blank reagent vial has been inserted into the Instrument it must not be turned, moved or removed until the calibration procedure is complete for all pipettes to be calibrated using that vial.

- 10.10 Enter the pipette serial number. Enter the volume to be checked. Once entered, the instrument will indicate which range solution should be used for that calibration.
- 10.11 Locate the appropriate sample solution in the reagent kit. Mix the contents by inverting the bottle several times before removing the cap. Recap the bottle as soon as possible to avoid evaporation.

**IMPORTANT:** Each kit contains bottles of sample solution corresponding to four different pipette volume ranges:

Range 1: For pipette volumes 200 to 5000  $\mu\text{L}$ .

Range 2: For pipette volumes 50 to 199  $\mu\text{L}$ .

Range 3: For pipette volumes 10 to 49  $\mu\text{L}$ .

Range 4: For pipette volumes 2 to 9  $\mu\text{L}$ .

**YOU MUST USE THE PROPER RANGE OF SOLUTION FOR THE PIPETTE VOLUME YOU ARE CALIBRATING.** If the improper sample solution has been pipetted into a blank reagent vial, that vial is no longer usable and should be discarded.

- 10.12 Transfer an aliquot of sample solution, using a disposable transfer pipette, into a sample aliquot container. Transfer pipettes and sample aliquot containers are found in the reagent kit. Place the container into the aliquot container holder to avoid any possibility of spillage. Place the cap lightly on the container to minimize evaporation.

**NOTE:** Transfer only enough reagent to be completely used in half-hour increments. The total volume of sample solution required depends on the pipette volume, the total number of pipettes tested and the total number of discrete data points being generated. The volume graduations on the pipette as well as the 1 mL marks on the sample aliquot containers can be used as rough guide.

- 10.13 Using the pipette to be calibrated, aspirate an aliquot of sample solution from the sample aliquot container. Use your standard pipetting



technique. Place the cap lightly back on the sample aliquot container between sampling to avoid errors due to reagent evaporation.

NOTE: When verifying the accuracy of a pipette there is an inevitable linkage between the operator and pipette. For meaningful results, it is recommended that you use your standard laboratory pipetting technique and follow your specific pipette manufacturer's instructions.

- 10.14 Lift the Instrument vial holder cover and dispense the aliquot of sample solution into the blank reagent vial.

IMPORTANT: The sample solution may be dispensed directly into the liquid or against the inside wall of the vial. If dispensing against the inside wall, the pipette tip should be placed above the meniscus but below the top of the vial holder. The mixing action of the PCS will wash the solution off the inside wall of the vial.

- 10.15 Once the aliquot is dispensed, carefully withdraw your pipette and close the cover. The PCS will display the results of the previous reading and will prompt you to add the next sample. Follow the same above procedure for sample #2 and for each subsequent sample addition. After each new sample addition, the instrument will mix, read and print the delivered volume.

- 10.16 When you have dispensed the desired number of samples for the calibration run (see Table 1), press the end of run button. The group statistics will be computed and the results printed.

- 10.17 Within a vial's allowed capacity, you may continue with additional calibration runs. You may perform calibrations all at the same sample volume, at lower or higher sample volumes but within the same range solution, or at lower or higher sample volumes and with different range solutions.

IMPORTANT: In calibrating pipettes from more than one range in a given vial, always use the range of sample solution which is appropriate to the volume of pipette being calibrated.

- 10.18 In calibrating an adjustable volume pipette at multiple volumes, you may start anywhere along its volume range. When you have dispensed the

desired number of samples at the first volume, press "Enter" (do not press "End of Run"). The group statistics for the first volume will be computed and the results printed. Follow the on screen instructions and indicate the next volume to be calibrated when prompted. The instrument knows that you are calibrating the same pipette but at a different volume. You may continue to calibrate the same pipette at different volumes by pressing the "Enter" key at the conclusion of each volume. Be sure to pay strict attention to the range solution to be used for a particular volume. After pipetting the final sample at the last volume for the pipette press the end of run key. The group statistics and results for the last volume and the footer will be printed.

11.0 DATA ANALYSIS/CALCULATIONS: None.

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in locked file cabinets in file room D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate forms (see 16.0).

13.3 To provide maximum life and dependable system performance, periodic cleaning should be performed. Refer to Chapter 7, Section 7.3, of the PCS Procedure Guide for instructions on cleaning the cabinet, the vial holder, and the filer wheel assembly.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

- 14.1 Acceptable limits for PCS Instrument Calibration are listed in the PCS 2 Pipette Calibration System Procedure Guide (see ref. 15.1). If Noise, Accuracy, Inaccuracy Due to Noise, Overall System Inaccuracy, or Overall Instrument Performance fail, clean the calibration vials and perform the instrument calibration check again. If the instrument fails again, purchase another Calibration set and recheck the calibration. If failures persist, schedule service for the instrument. Do not use the instrument for calibration until it has been recertified.
- 14.2 Do not use pipettes if the inaccuracy exceeds  $\pm 5\%$  of the target volume.
- 14.3 See Chapter 7, Troubleshooting, of the PCS Procedure Guide for specific system performance checks. Section 7.2 provides a troubleshooting guide for symptoms, causes, and corrective actions.

15.0 REFERENCES:

- 15.1 Artel, Inc. 1997. PCS 2 Pipette Calibration System Procedure Guide, Doc #: 15A2135 Ver: 5.1, 03/28/97.

16.0 FORMS AND DATA SHEETS:

- 16.1 Pipette Calibration Record Sheet
- 16.2 Sample PCS Printout

## Pipette Calibration Record Sheet

### OPP Microbiology Laboratory

PIPETTE CALIBRATION SUMMARY			
Test Start Time		Test Stop Time	
Date		Volume Tested	
Initials		Range Solution	
Pipette Vol. Range		Replicates	
Serial No.		Reagent Lot No.	
Sample #	Volume	Mean	
1		Standard Dev.	
2		%CV	
3		Inaccuracy	
4		Pass/Fail	
5		Nonconformance	
Comments:			

PIPETTE CALIBRATION SUMMARY			
Test Start Time		Test Stop Time	
Date		Volume Tested	
Initials		Range Solution	
Pipette Vol. Range		Replicates	
Serial No.		Reagent Lot No.	
Sample #	Volume	Mean	
1		Standard Dev.	
2		%CV	
3		Inaccuracy	
4		Pass/Fail	
5		Nonconformance	
Comments:			

Sample PCS Printout  
OPP Microbiology Laboratory

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US EPA / MARYLAND  
FT. MEAD, MD 20755

DATE: 06 Nov 00  
TIME: 13:03

PIPETTE CALIBRATION

PIPETTE SERIAL #: 152348  
OPERATOR ID: 1  
REAGENT LOT #: 14758  
TEMPERATURE (C): 22.1

PIPETTE SAMPLE VOLUME: 10.00 uL  
SAMPLE RANGE: 3

SAMPLE #	VOLUME
1	9.94
2	9.97
3	9.94
4	10.03
5	9.98

NO.PTS: 5  
MEAN: 9.97  
STD: 0.037  
%CV: 0.37%  
INACC: -0.28%

LAST INST. CALIBRATION CHECK: 27 Oct 00  
SOFTWARE VERSION: 6.013  
INSTRUMENT SERIAL #: 6250  
ARTEL PCS , PATENTS PENDING

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